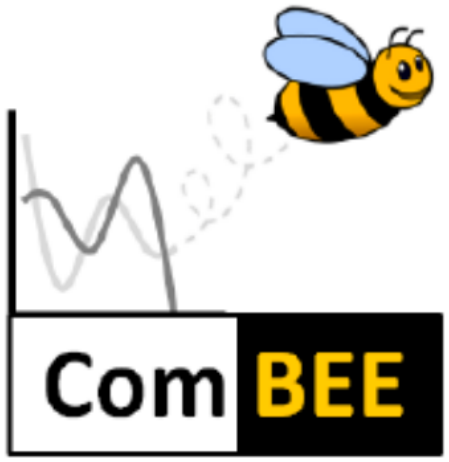
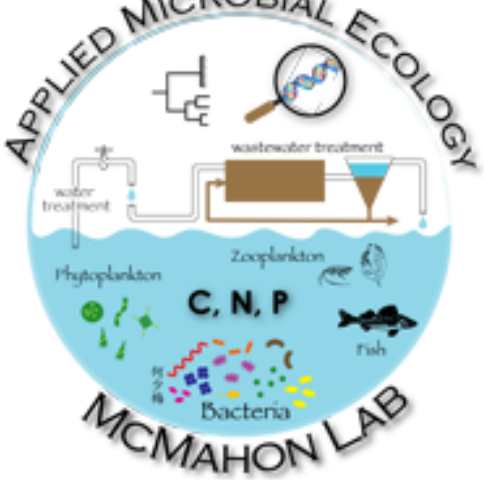
ComBEE ‘Omics Study Group: Phylogenetic Analysis

Benjamin Peterson





What is phylogeny?

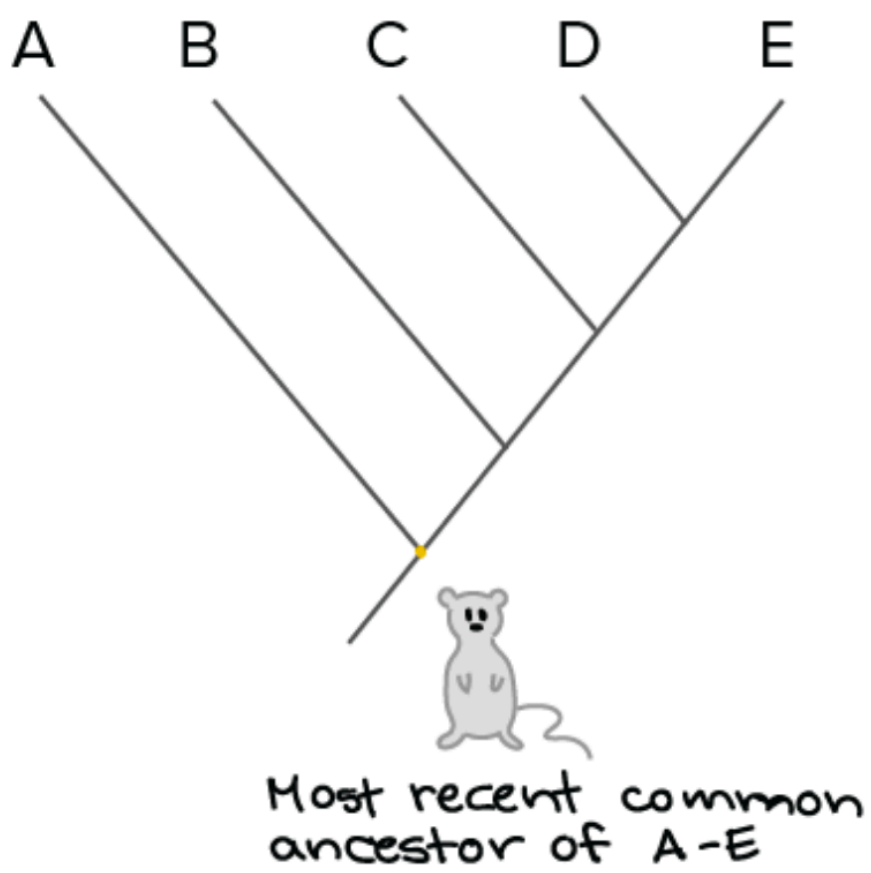
Phûlon – “genus”, “species”, “tribe”

-géneia – “generation”

What is phylogenetic inference?

- A hypothesis regarding the evolutionary relationship between units of life

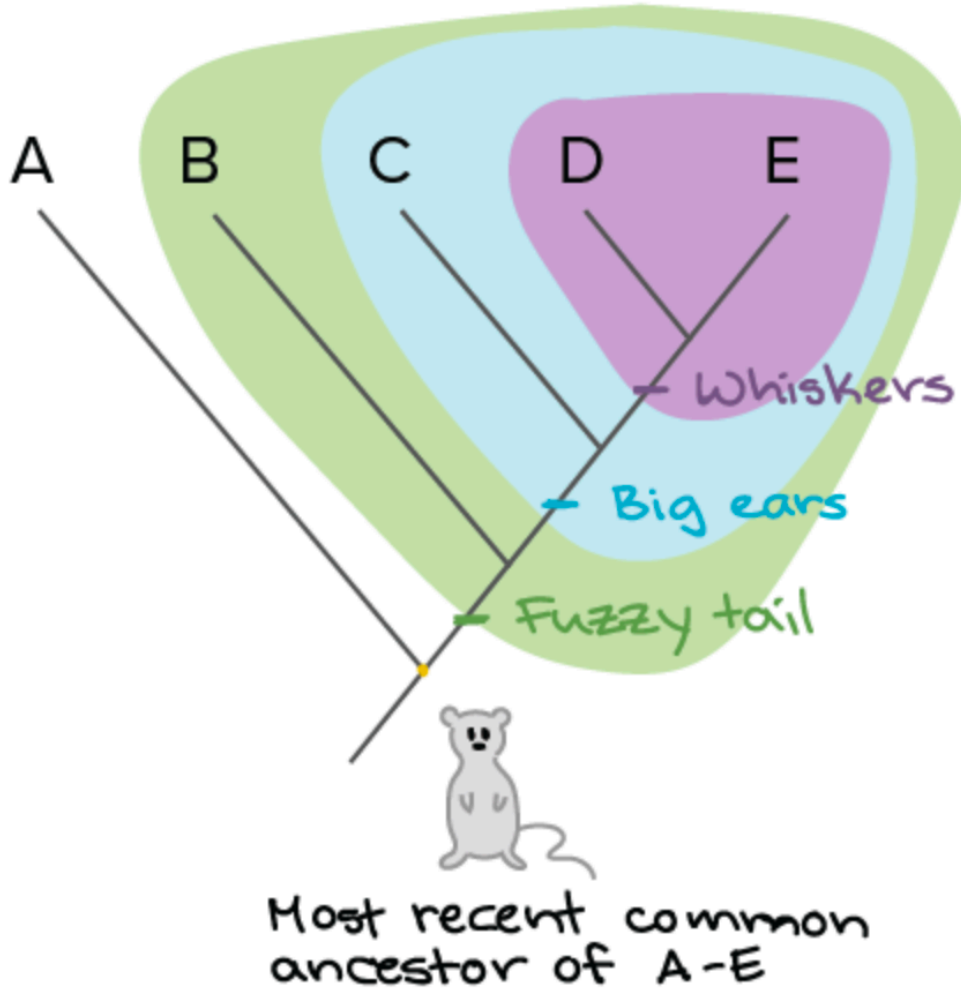
- What is this based on?



https://www.khanacademy.org/science/biology/her/tree-of-life/a/building-an-evolutionary-tree

What is phylogenetic inference?

- A hypothesis regarding the evolutionary relationship between units of life
- Primarily based on “derived traits”



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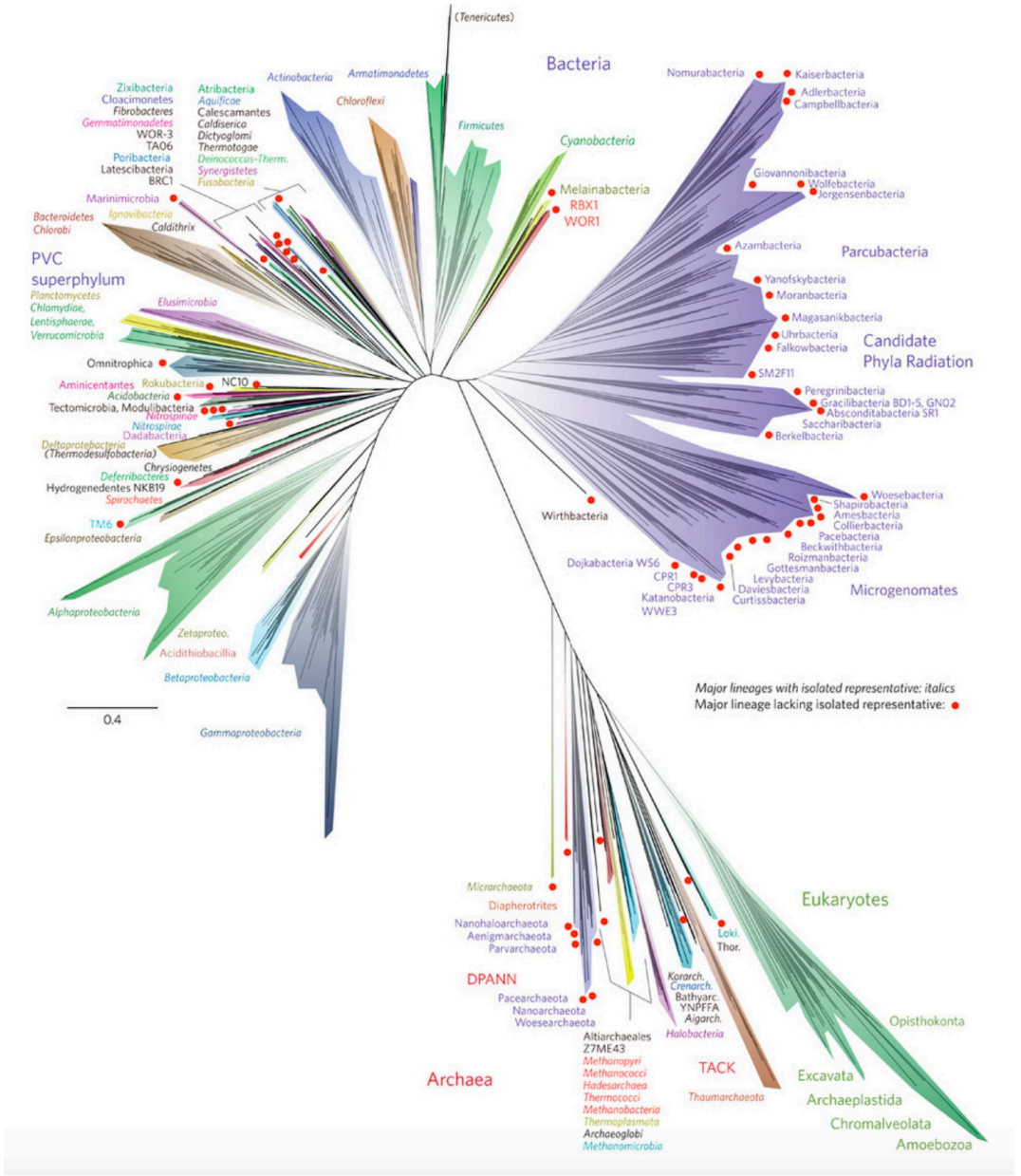
Molecular phylogenetics

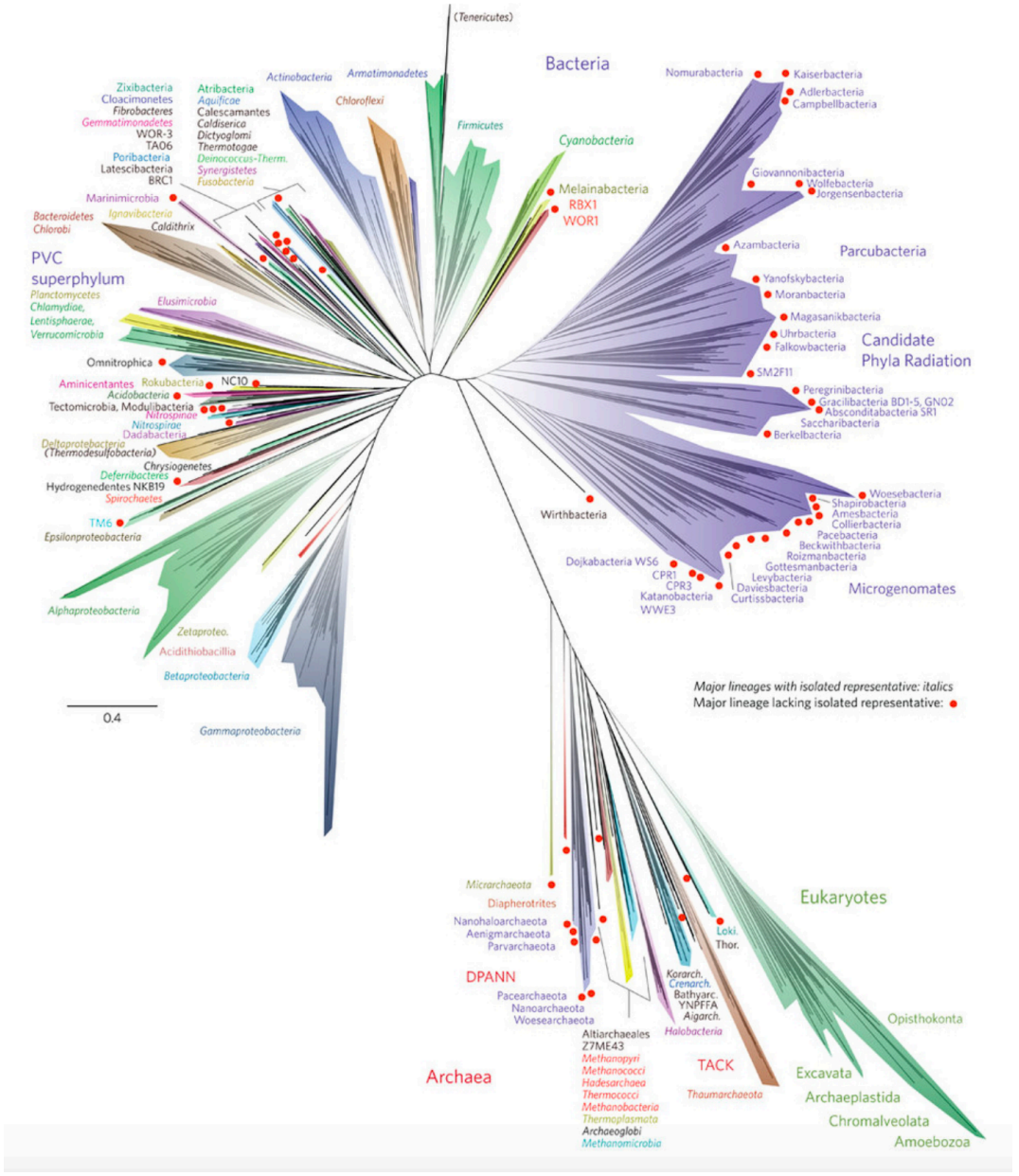
- What are the derived traits?



Hug et al, 2016

Molecular phylogenetics

- Substitutions in sequences (nucleic acid or amino acids) are the “derived traits”
- Provide direct insight into actual evolutionary processes

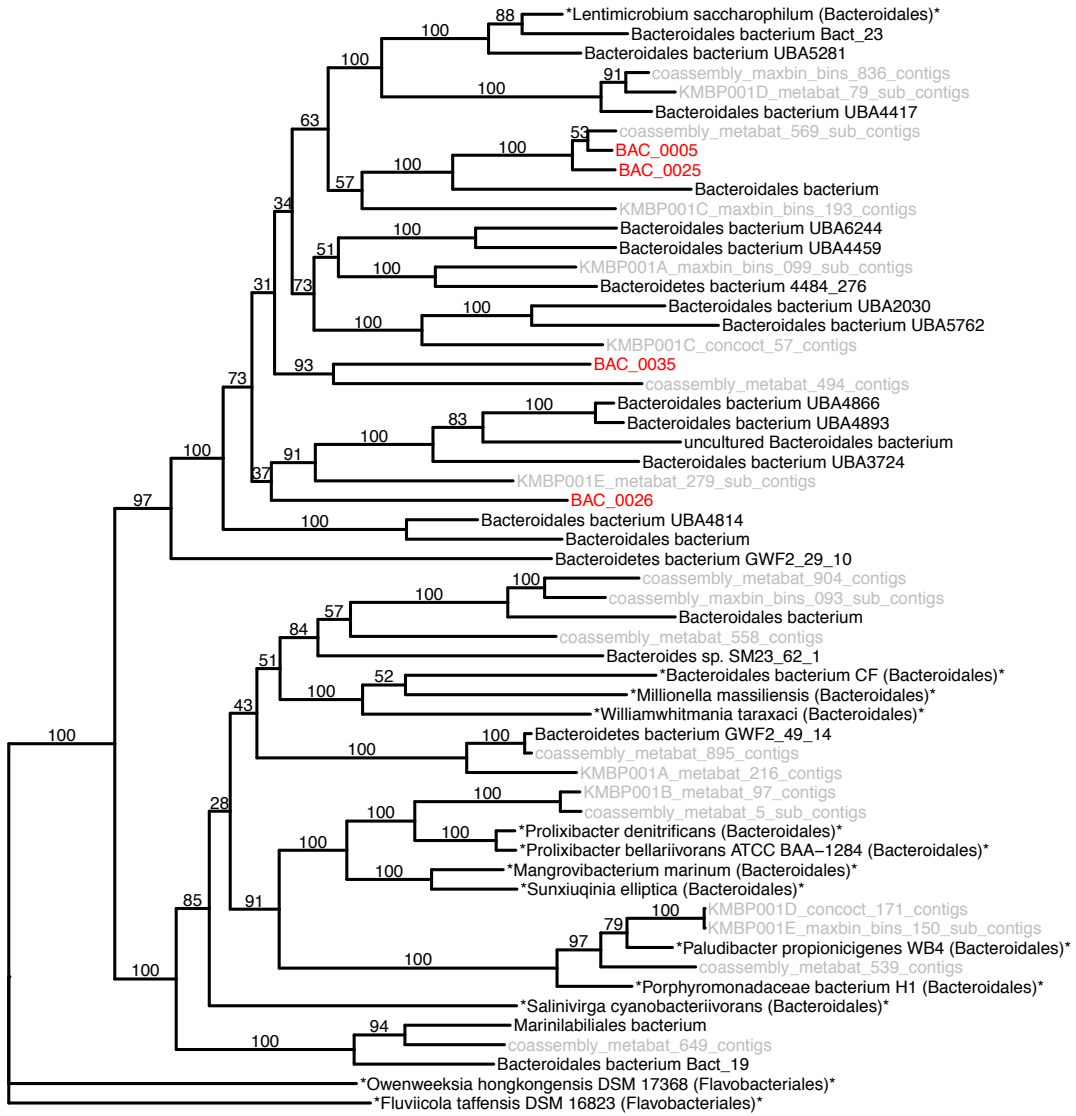


Hug et al, 2016

Uses for phylogenetic inference

Uses for phylogenetic inference

- Taxonomic classification
- Evolutionary relationships of organisms/genes
  - Searching for horizontal gene transfer
- Estimate of functional classification of genes
- ?????????



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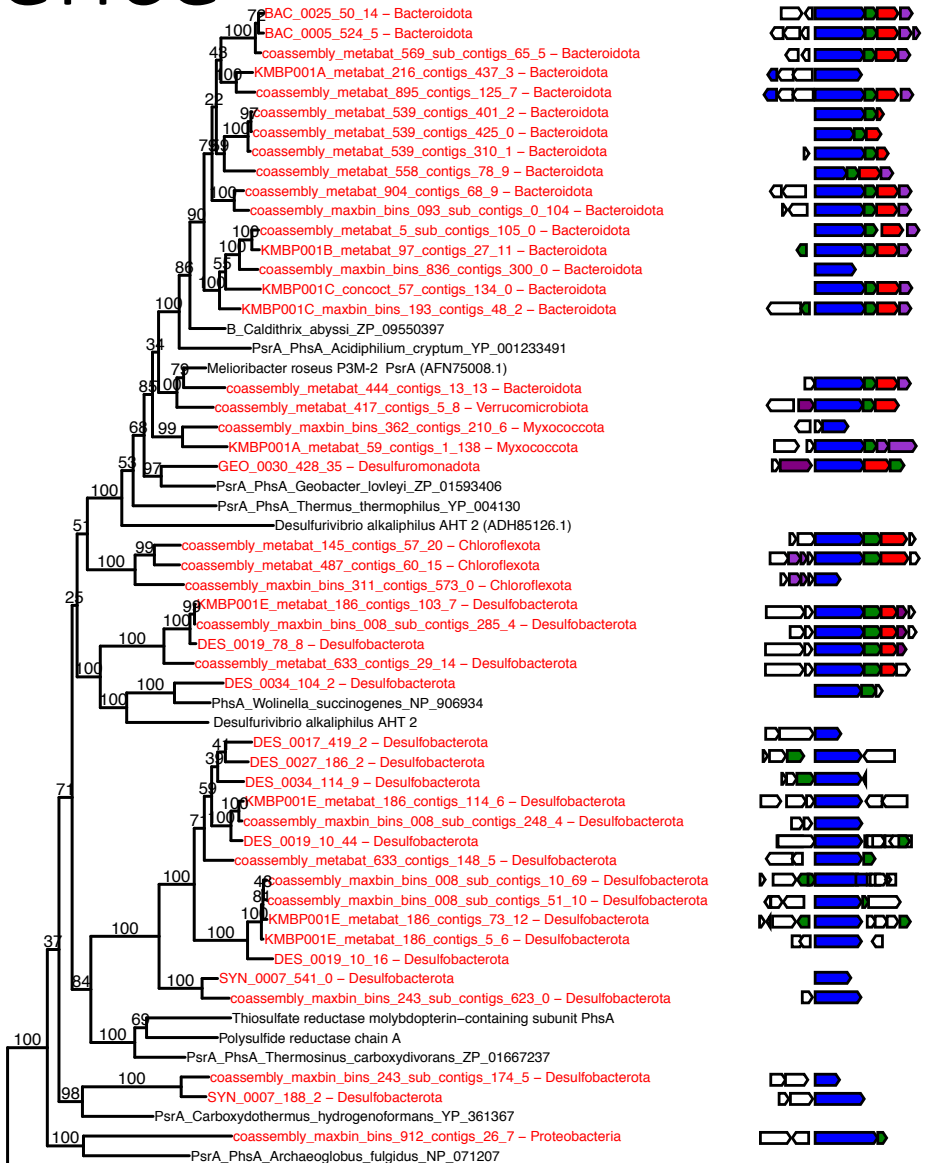
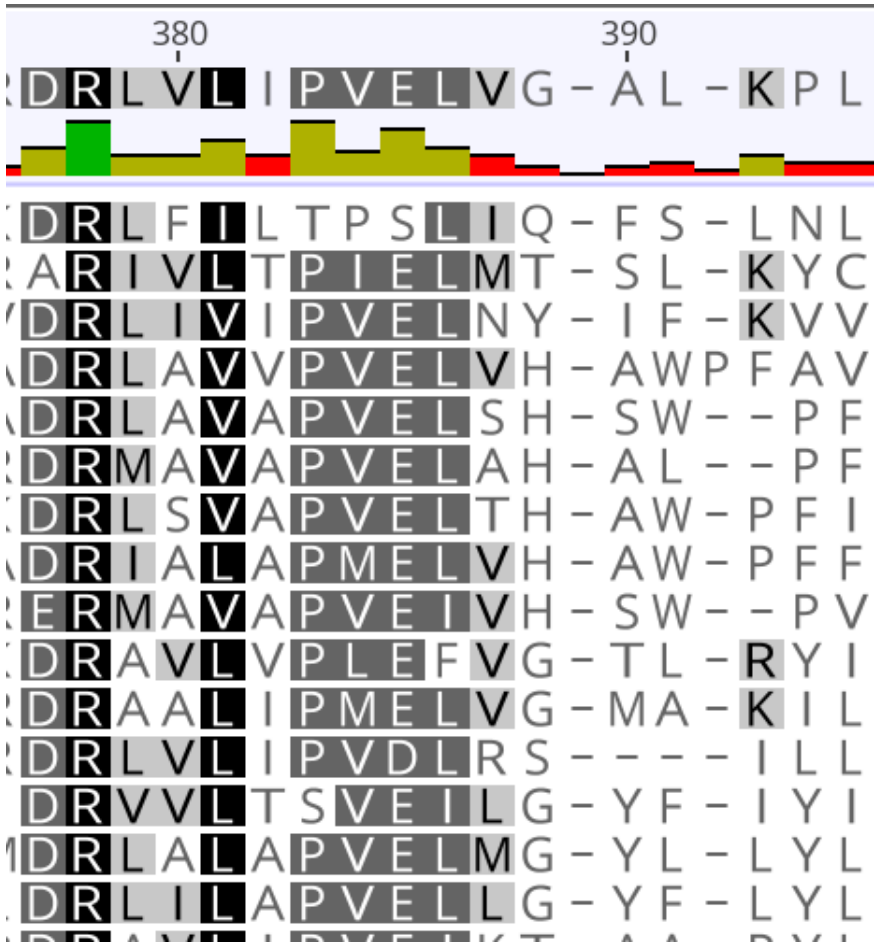


Steps in building a phylogenetic tree

1. Basic workflow
    1. Selection of sequence to use
    2. Generate sequence alignment
        1. Alignment masking and trimming
    3. Generate phylogenetic tree
    4. Check branch support and tree quality
    5. Root tree (if needed)
2. Potential modifications
    1. Removal of divergent sequences

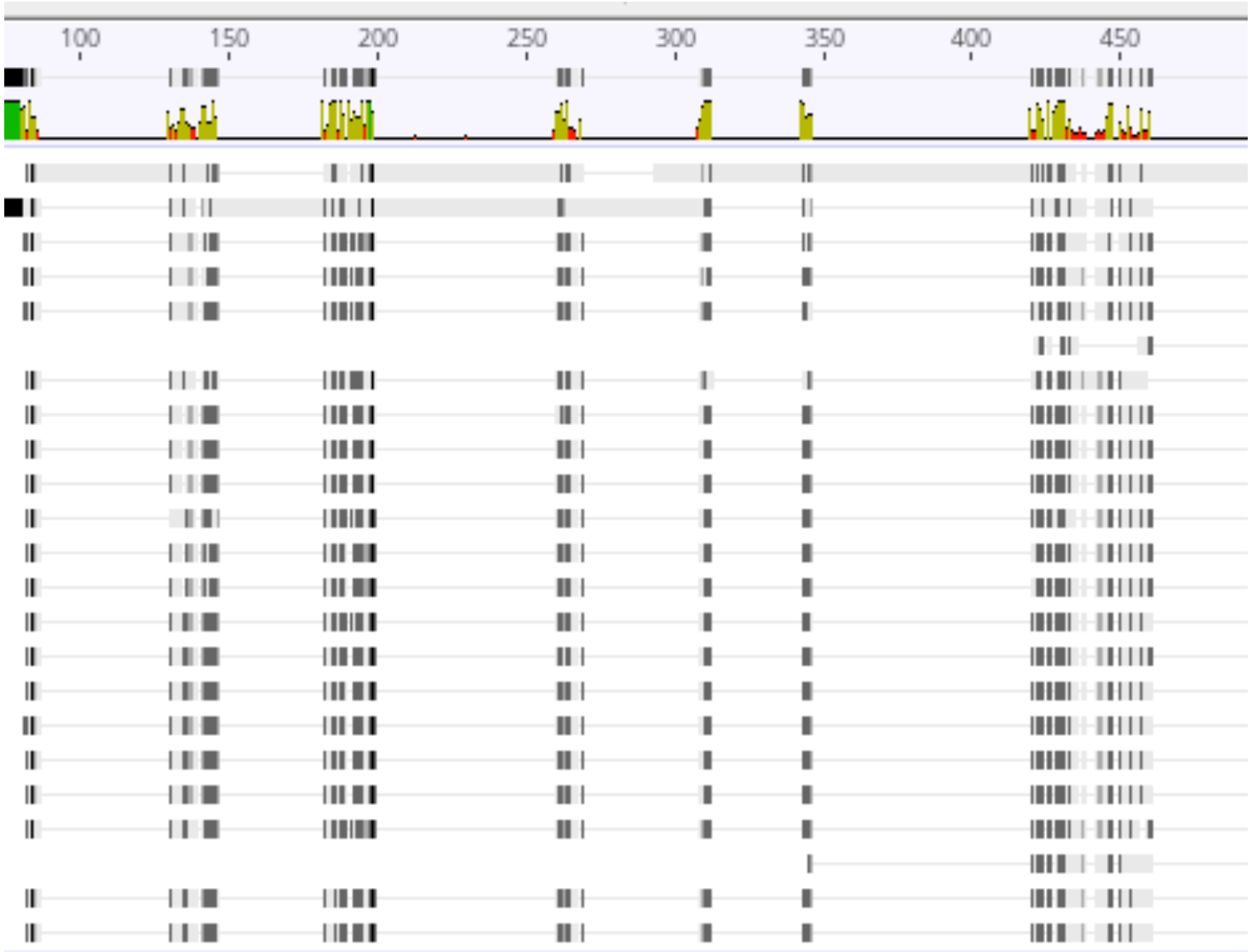
Generation of sequence alignment

1. Multiple sequence alignment
    1. MUSCLE
    2. Clustal
    3. T-Coffee
    4. MAFFT



Generation of sequence alignment

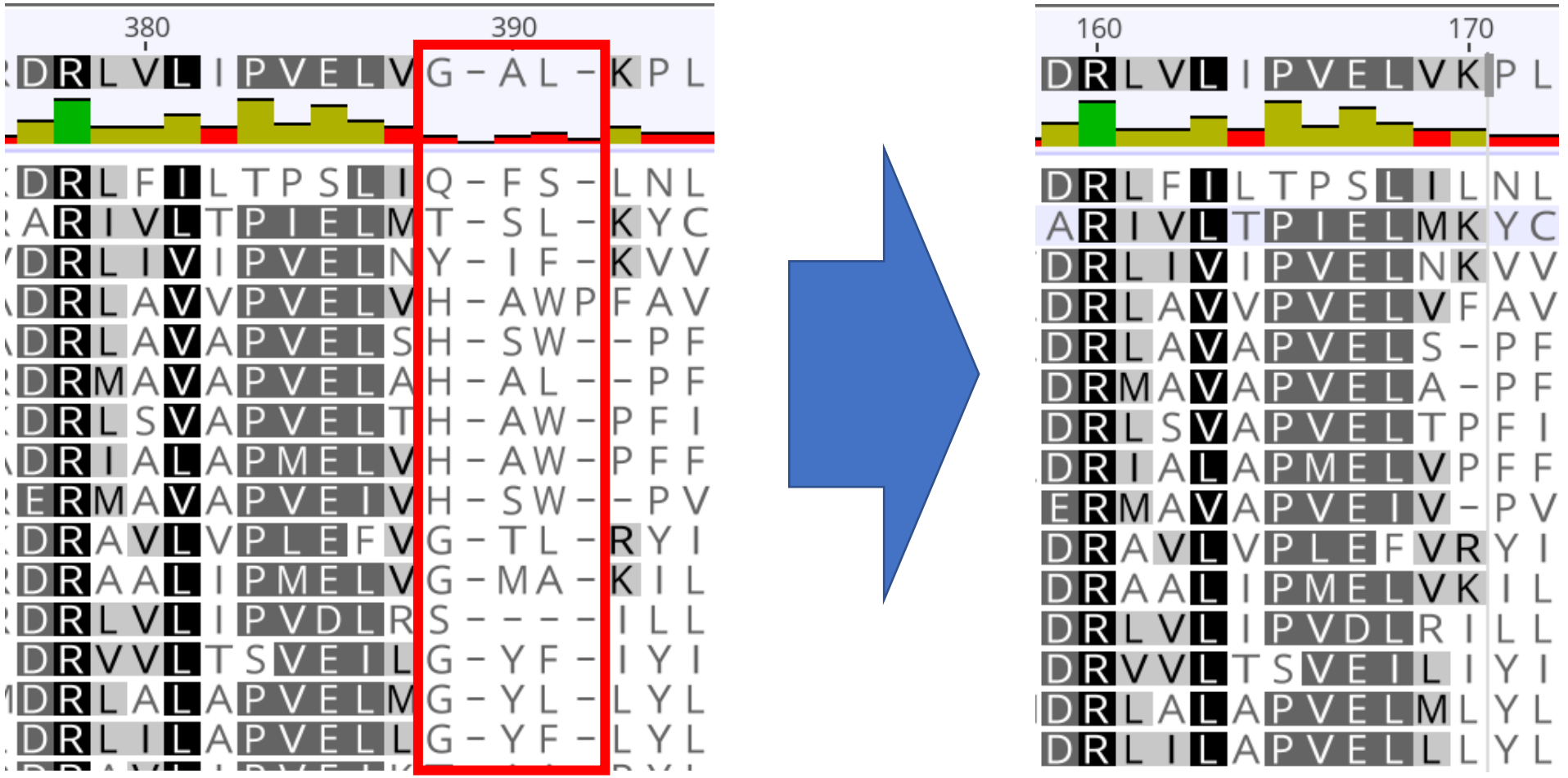
1. Manual inspection of alignment
    1. Do some sequences need to be removed?



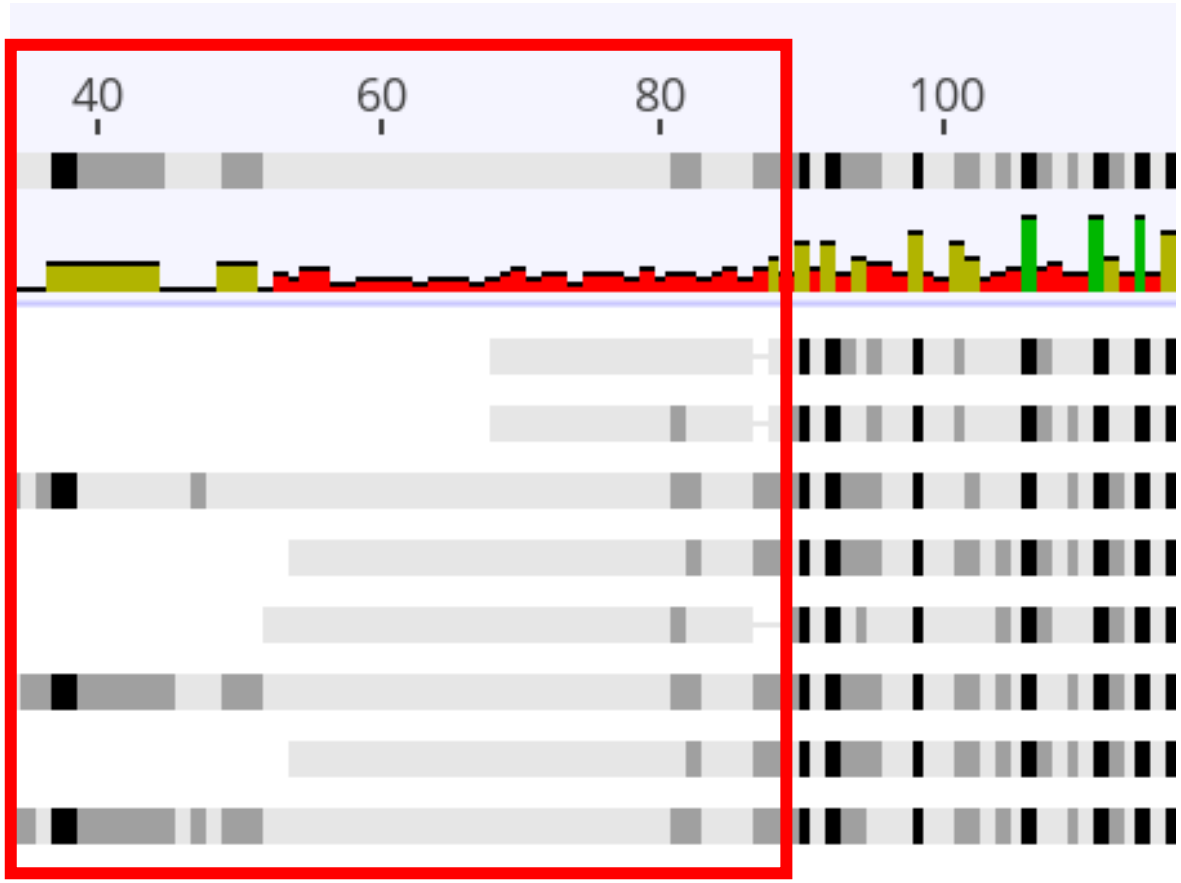
Masking Methods

1. Trimming strategies
    1. Fraction of gaps and/or trim overhanging ends
    2. Modeling suitability using substitution matrices (BMGE1.1)

Masking an alignment



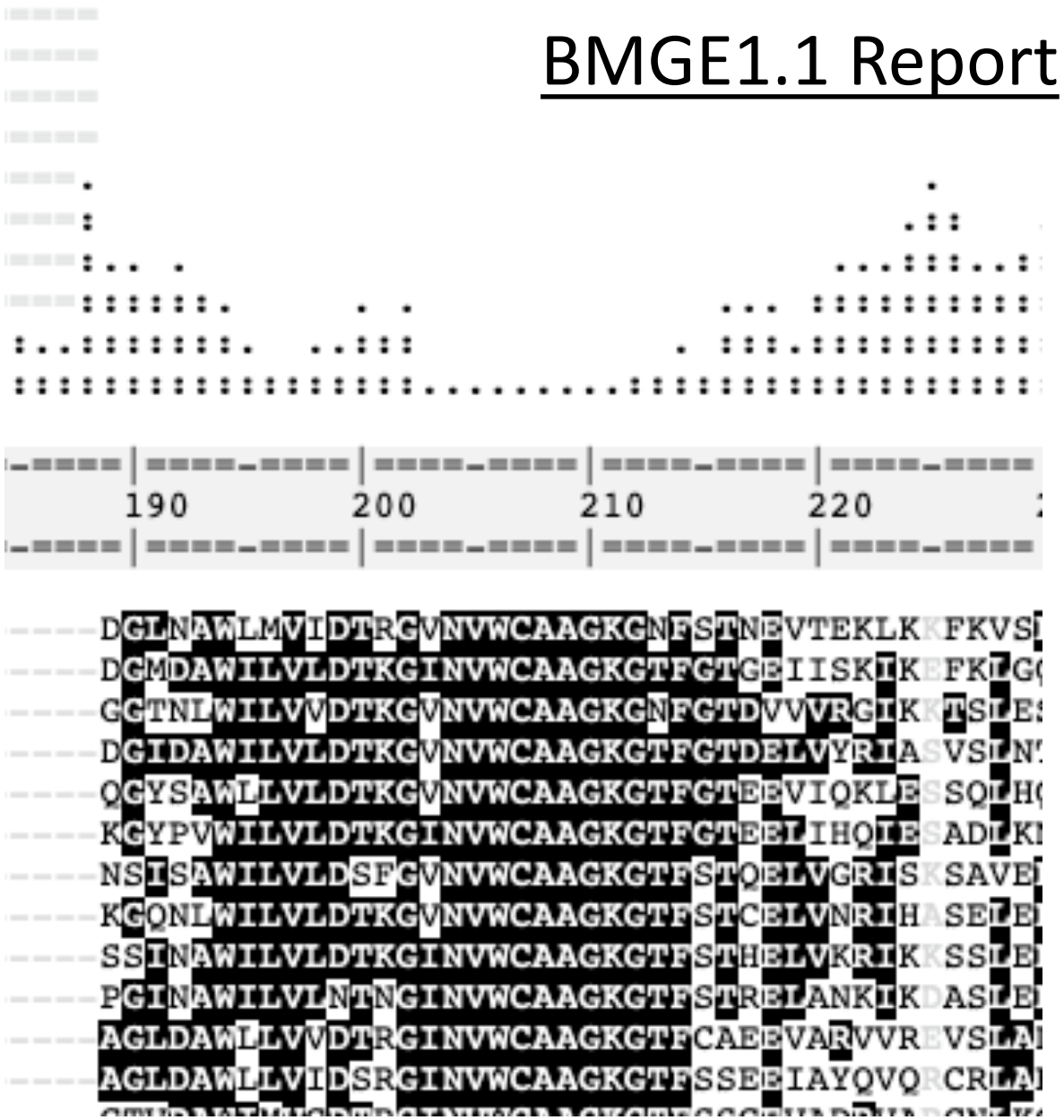
Trimming ends of alignment



Masking Methods

1. Trimming strategies
    1. Fraction of gaps
    2. Overhanging ends
    3. Modeling suitability using substitution matrices (BMGE1.1)

BMGE1.1 Report



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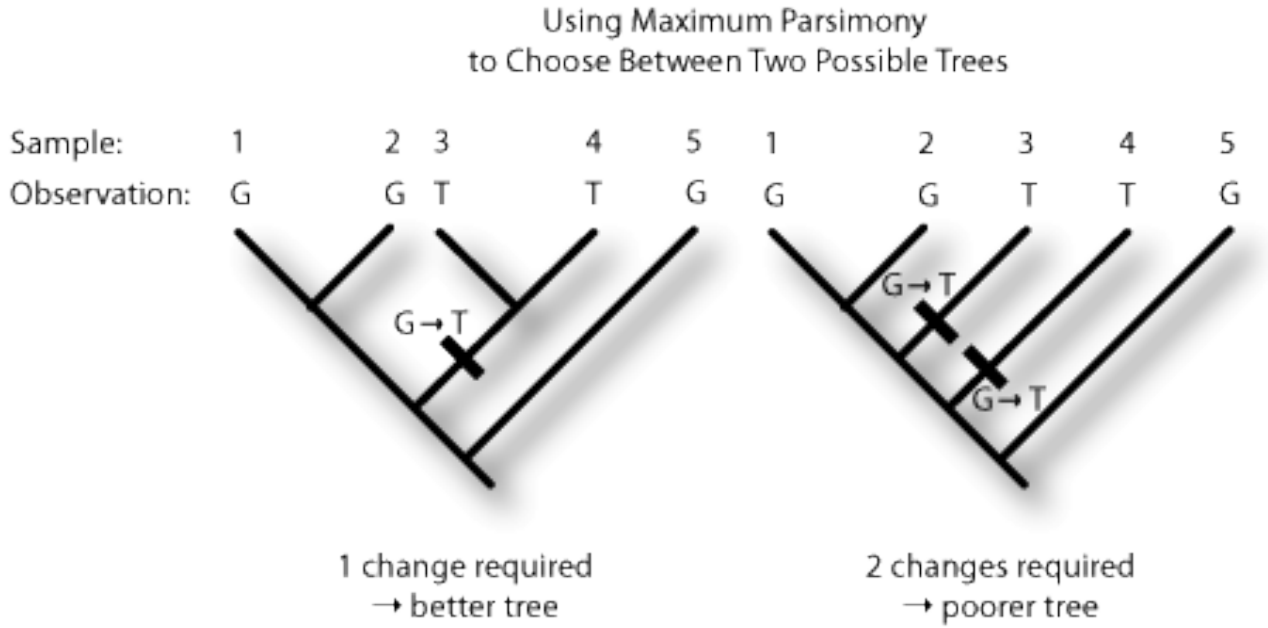
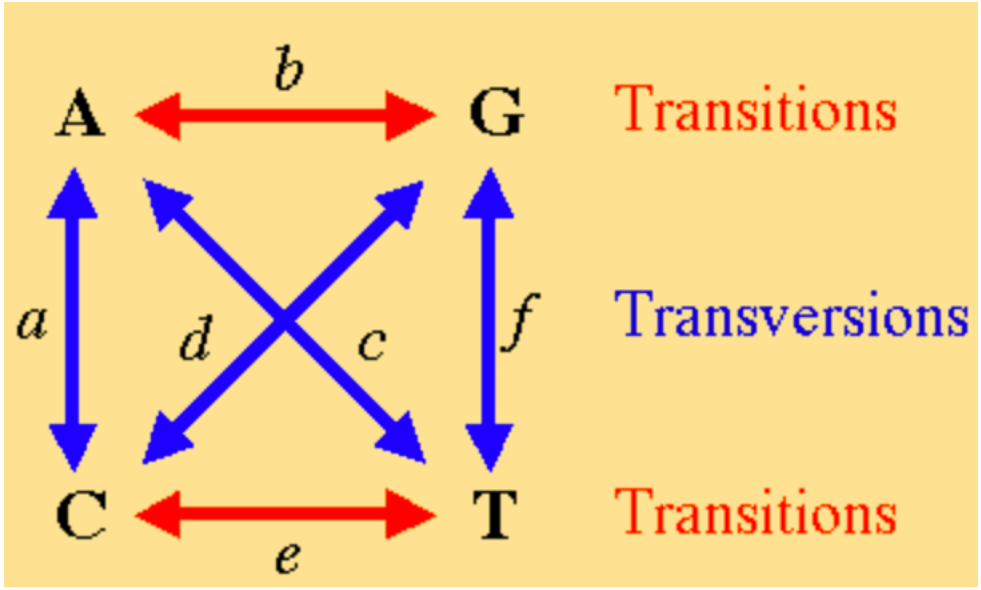
Common methods of tree generation

1. Maximum Parsimony
   1. No model for evolution
   2. Tries to minimize number of changes in sequence
   3. Not often used these days



Molecular models of evolution - DNA

1. Need to account for likelihood of shifts in sequences
    1. Jukes-Cantor assumes all are equally likely
    2. Others have differential rates



http://artedi.ebc.uu.se/course/X3-2004/Phylogeny/Phylogeny-Criteria/Phylogeny-Criteria.html

Molecular models of evolution - proteins

1. Protein substitutions are significantly more complicated
    1. Many different models: LG, WAG, BLOSUM, etc etc

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

https://upload.wikimedia.org/wikipedia/commons/0/02/BLOSUM62.png

Common methods of tree generation

1. Distance methods
    1. Quick, useful for checking tree
    2. Generates distance matrix from alignment
    3. Neighbor-joining method
2. Maximum Likelihood
    1. Computationally intensive, but shortcuts can be made
    2. Generates multiple optimized trees and compares to model-predicted score
    3. RAxML or FastTree (approximately)
3. Bayesian inference
    1. Computationally intensive, complex, requires accurate prior probabilities
    2. Returns actual probability of data being correct (based on priors)
    3. Allows for incorporation of prior knowledge
    4. Mr. Bayes

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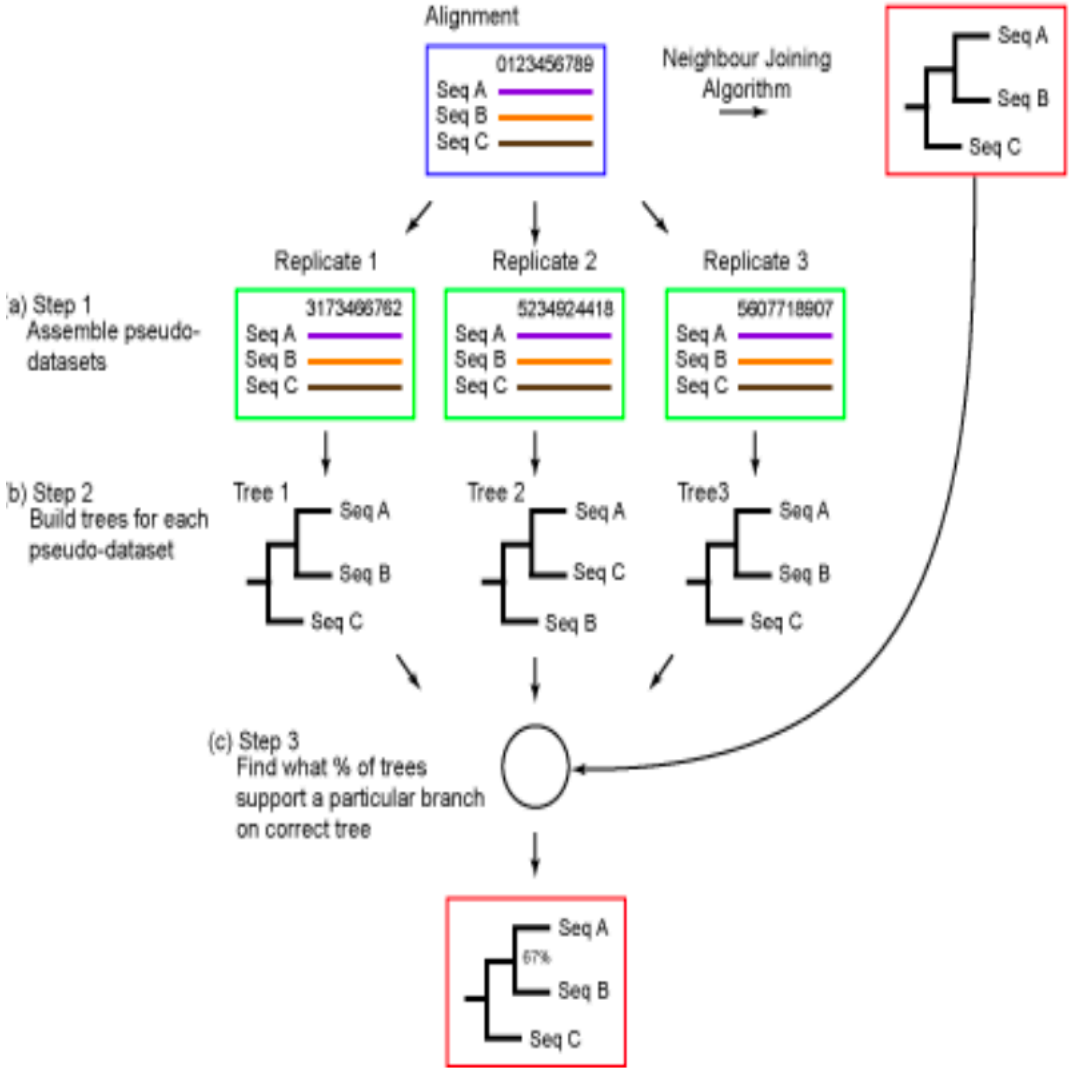
Branch support methods - Bootstrapping

1. Random subsampling of the alignment with replacement
2. Generation of tree based on new sequences
3. Repeat many times
4. Calculate fraction of trees that have a particular branch
5. **Is a branch label, not a node label**

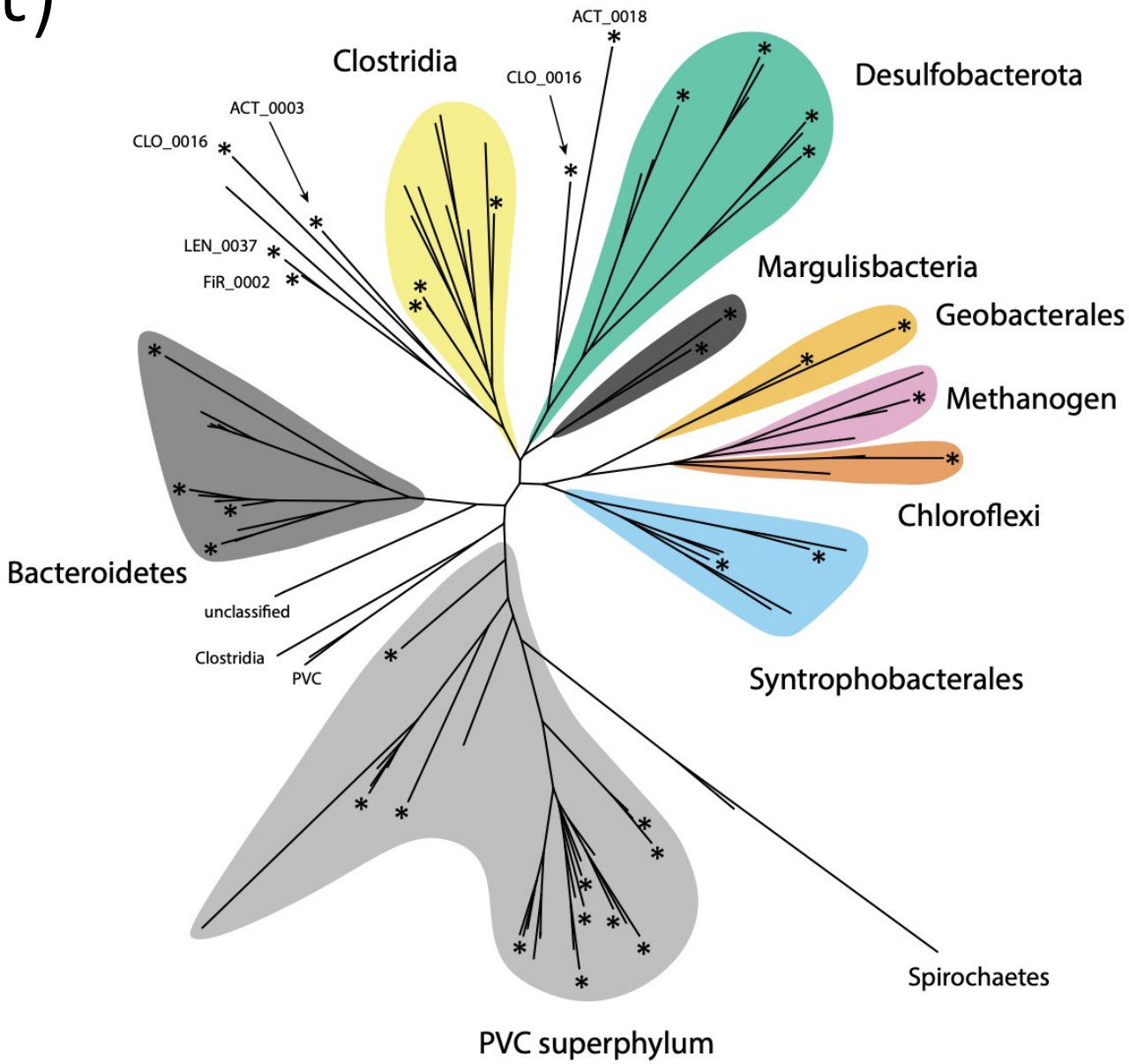


To root, or not to root?

1. Main points to consider:
    1. What is your question?
        1. Relatedness vs. evolution
    2. What information do you have about the sequences and their evolutionary history?

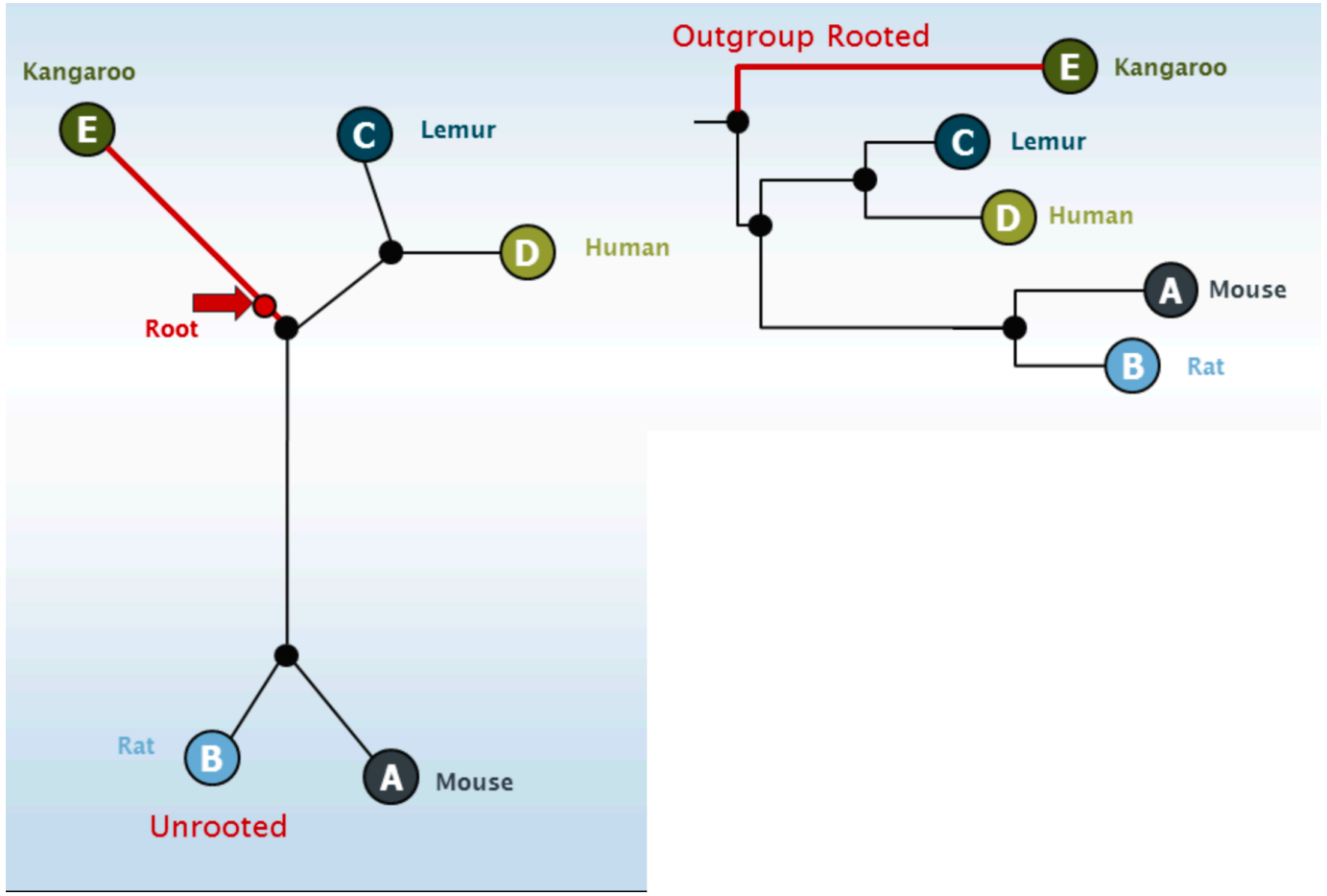
Tree rooting (or not)

1. Leave it unrooted
    1. Useful if just interested in the “similarity” or “relatedness”
    2. Can’t be used to discuss ancestral state or direction of evolution



Tree rooting (or not)

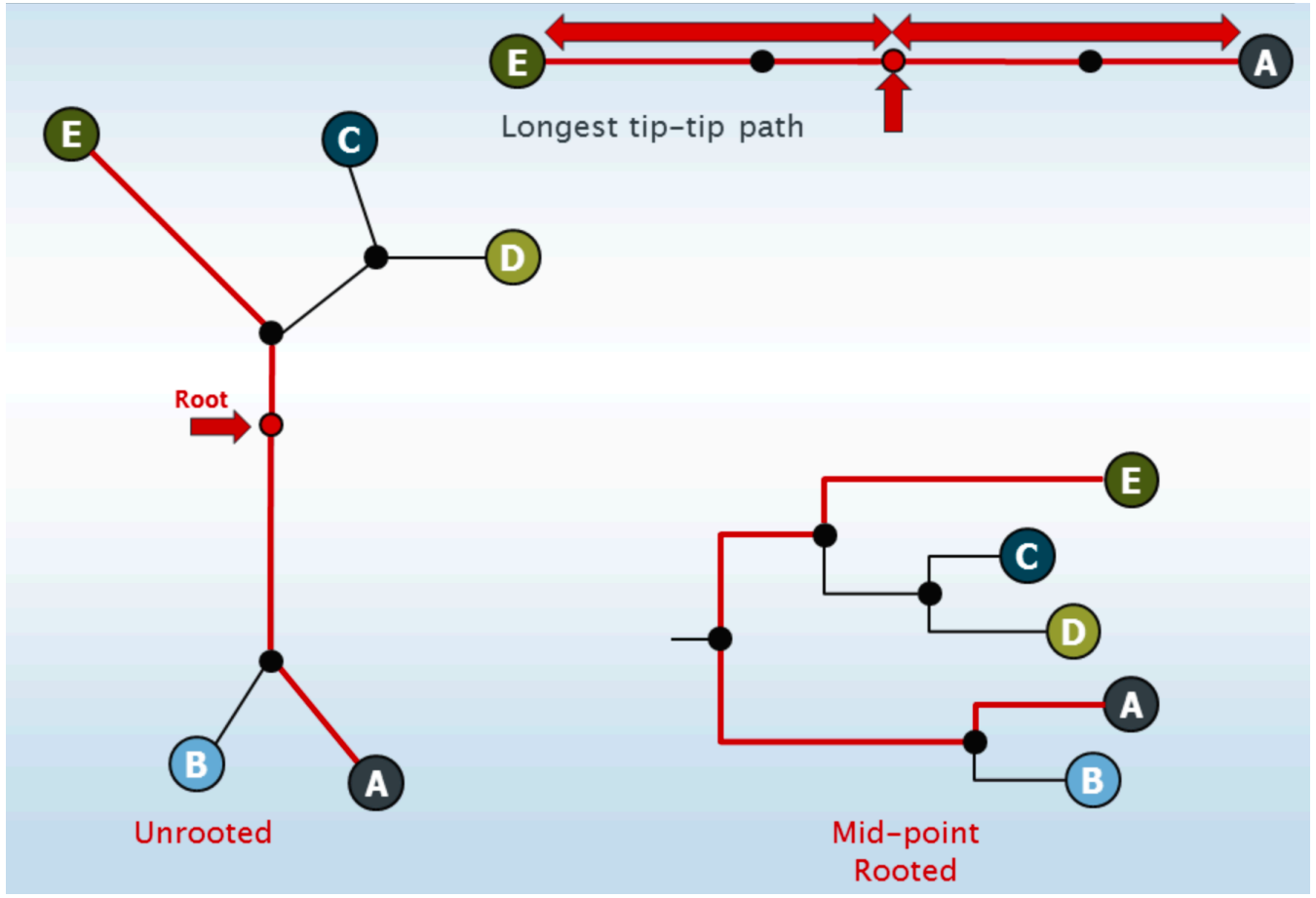
2. Outgroup rooting
    1. Assumes you know a true outgroup and that it's closely related enough
    2. Can include outgroups in the alignment and tree generation
    3. Or can generate tree without outgroup and place the outgroup on the tree later



http://cabbagesofdoom.blogspot.com/2012/06/how-to-root-phylogenetic-tree.html

Tree rooting (or not)

3. Midpoint rooting
    1. Assumes constant evolutionary rate
    2. Doesn't require *a priori* knowledge of outgroup



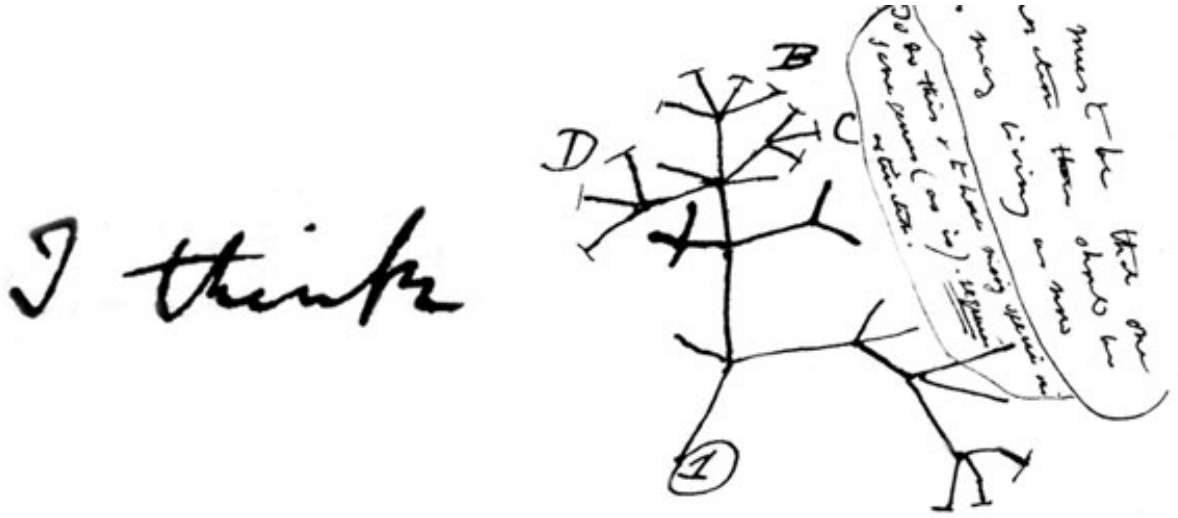
http://cabbagesofdoom.blogspot.com/2012/06/how-to-root-phylogenetic-tree.html

Reiteration of tree

1. Done if you want to improve bootstrapping values/posterior probabilities
2. Removal of highly divergent sequences
    1. Manual inspection or automatic (RogueNaRok)
3. Different masking techniques

References to check out

1. **Tree Thinking – David Baum and Stacey Smith**
2. Papers by Alexi Stamatakis
3. Molecular phylogenetics: principles and practice (review paper)
4. http://phylobotanist.blogspot.com/2015/01/how-to-root-phylogenetic-tree-outgroup.html
5. http://cabbagesofdoom.blogspot.com/2012/06/how-to-root-phylogenetic-tree.html



Darwin's Tree